M-MLV (H-)Reverse Transcriptase

M-MLV	50 µl
5X first-strand Buffer	200 µl

For research use only
Cat No: YT4502
Size: 10000 U (200U/µl)
Store at –20°C

Description :

Moloney murin leukemia virus reverse transcriptase is a DNA polymerase which uses a single stranded RNA or DNA as a template. This enzyme synthesized complementary DNA strand in the presence of primers. This enzyme is isolated from E.coli expressing a portion of the pol gene of MMLV on plasmid.it shows optimal activity around 37C .the enzyme is capable to synthesized full length cDNA (up to 5kb) from almost any transcript. The enzyme lacks DNA endonuclease activity.

Unit Definition:

One unit incorporates 1 nmole of dTTP into acid-precipitable material in 10 min at 37°C using poly(A) •oligo(dT) 25 as template-primer.

Storage Buffer

20mM Tris-HCl (pH 7.5), 200mM NaCl, 0.1mM EDTA, 1mM DTT, 0.01% NP-40, 50% glycerol

5xfirst-Strand Buffer

250mM Tris-HCl (pH 8.3 at 25°C), 375mM KCl,15mM MgCl2 50mM DTT

Applications:

First-strand cDNA Synthesis

Protocol:

First-Strand cDNA Synthesis Using M-MLV RT

A 20-µl reaction volume can be used for 1ng–5µg of total RNA or 1–500ng of mRNA.

1. Add the following reagents into a sterile, nuclease-free nuclease-free tube on ice in the indicated order:

Template RNA	Poly (A) mRNA Or specific RNA	1 to 500 ng 1-5 µg
Prime	oligo (dT)18 primer(50µM) or Random hexamer primer(50µM)	1 µl 1 µl
DEPC-treated water		To 13.5 µl
Total Volume		13.5 µl

2. Mix gently, centrifuge briefly and incubate at 70°C for 5 min. Chill on ice, spin down and place the vial back on ice.

3. Prepare the following cDNA Synthesis Mix, add the following components in the indicated order:

5x first-strand buffer	4 µl
dNTPs(10 mM each)	1 µl
RNasin (40U/µl)	0.5 µl
M-MLV	1 µl

4.Mix gently and centrifuge
5.For oligo(dT)18, incubate for 60 min at 42°C. For random hexamer primed synthesis, incubate for 60 min at 37°C .
6.Terminate the reaction by heating at 70°C for 5 min. The reverse transcription reaction product can be used immediately in second strand cDNA synthesis reactions or stored at -20°C for less than a week. For longer storage, -70°C is recommended.